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(54) Title: CARCINOMA TREATMENT

(57) Abstract

For the treatment of carcinomas in epithelial cells and for the prevention of epithelial cell proliferation, use is made of a CD40 receptor binder. The CD40 may be used in conjunction with at least one antineoplastic agent and/or CD40 inducer.

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CARCINOMA TREATMENT

This invention relates mainly to carcinoma treatment and is more particularly concerned with the treatment of carcinomas in epithelial cell systems, although the present invention, in its broadest aspect, relates generally to the prevention of epithelial cell proliferation.

It is known to treat carcinomas in epithelial cell systems using anti-neoplastic agents which are intended to induce apoptosis. Examples of such agents include cytokines and growth factors such as transforming growth factor β_1 (TGF-β_1), anti-Fas, and tumour necrosis factor-α (TNF-α) and other antineoplastic agents such as interferon-γ (IFN-γ), *cis*-platin, mitomycin C, cyclophosphamide, actinomycin D, doxorubicin, vincristine, etoposide, 5-fluorouracil, methotrexate and irradiation agents. However, there is a continuing need for other drugs for treatment of carcinomas in epithelial cell systems and/or for enhancing the effect of existing anti-neoplastic agents.

In a first aspect, the present invention resides in the use of a CD40 receptor binder for the manufacture of a medicament for the prevention of epithelial cell proliferation or for the treatment of carcinomas in epithelial cell systems.

In a second aspect, the present invention resides in the use of a CD40 receptor binder in the manufacture of a medicament for enhancing the susceptibility of neoplastic epithelial cells to anti-neoplastic drug-induced apoptosis.

In a third aspect, the present invention resides in the use of a CD40 receptor binder in combination with a CD40 inducer for the prevention of epithelial cell proliferation or for the treatment of carcinomas in epithelial cell systems.

In a fourth aspect, the present invention resides in the use of a CD40 receptor binder in combination with an anti-neoplastic drug for the treatment of carcinomas in epithelial cell systems.

In a fifth aspect, the present invention resides in a method of preventing epithelial cell proliferation, comprising administering a CD40 receptor binder to a human or animal patient.

In a sixth aspect, the present invention resides in a method of treating an epithelial cell carcinoma in a human or animal patient, comprising administering a CD40 receptor binder to the patient.

Preferably, such administration is effected in conjunction with administration of at least one anti-neoplastic agent and/or a CD40 inducer.

The anti-neoplastic agent may be selected from known cytokines and growth factors such as transforming growth factor β_1 (TGF-β_1), anti-Fas, and tumour necrosis factor-α (TNF-α) and other known antineoplastic agents such as interferon-γ (IFN-γ), *cis*-platin, mitomycin C, cyclophosphamide, actinomycin D, doxorubicin, vincristine, etoposide, 5-fluorouracil, methotrexate and irradiation agents. Such anti-neoplastic agent will normally be administered at a dose rate consistent with the recommended for that particular agent.

The CD40 inducer may be interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) or any other known inducer of CD40 expresssion. Such CD40 inducer will normally be used at a dose rate consistent with that recommended for that particular inducer.

CD40 is a 50-kDa type 1 glycoprotein belonging to the nerve growth factor receptor/tumour necrosis factor receptor (NGF-R/TNF-R) family, and is expressed on the surface of B lymphocytes (see Noelle et al., Immunol. Today, **13**, 431-433, 1992; and Smith et al., Cell, **76**, 959-962, 1994). For both normal and neoplastic B cells prone to enter or be driven into apoptosis, ligation of CD40 provides a potent rescue (survival) signal which is partially mediated through the up-regulation of Bcl-2 expression (see Liu et al., Nature, **342**, 929-931, 1989). This effect was shown initially using ligand-mimetic monoclonal antibodies (mAb) and more recently confirmed with recombinant ligand (CD40L) (see Noelle et al., **supra**).

It is also known that expression of both CD40 and Bcl-2 occurs in normal, basal epithelial cells and in a number of different carcinomas including those of the ovary, nasopharynx, lung and breast (see Young et al., Int. J. Cancer, **43**, 786-794, 1989; and Hockenbery et al., Proc. Natl. Acad. Sci. USA, **88**, 6961-6965, 1991). These studies suggest that the CD40 pathway may be active in carcinomas and that the resulting expression of Bcl-2 may protect these tumours from apoptotic cell death including that induced by the cytotoxic drugs used in conventional cancer chemotherapy (see Dive et al., Semin. Cancer Biol, **3**, 417-427, 1992). Furthermore, the restricted expression of CD40 and Bcl-2 to the proliferative basal compartment of stratified, squamous epithelium

indicates a possible role for this pathway in regulating normal growth and the apoptotic process of epithelial differentiation.

In view of the above, it was surprising to find that, contrary to the studies in B cells, CD40 stimulation in epithelial cells of either normal or neoplastic origin results in growth arrest and enhanced susceptibility to anti-neoplastic agent-induced apoptosis.

As examples of CD40 binders, there may be used ligand-mimetic monoclonal antibodies, for example G28.5 (a hybridoma available under the Accession No. HB-9110 from the American Type Tissue Culture Collection, Rockville, Maryland, USA), S2C6 (as disclosed by Paulie et al, Cancer Immunology and Immunotherapy, 20, 23-28, 1985), and naturally occuring or engineered forms of CD40 ligands, of which an example is CD40L. CD40L is a 39-kDa type II integral membrane protein with homology to TNF, which can be induced on T cells following their activation via the T cell receptor (see Clark & Ledbetter, Nature, **367**, 425-428, 1994). The CD40-CD40L pair are part of an expanding family of interacting receptor-ligand molecules based on the TNF-R/TNF families which are involved in cellular activation and regulation of apoptosis in target cells (Smith et al., **supra**).

The CD40 binders may be administered by intravenous, subcutaneous or intraperitoneal injection or infusion, or by direct injection into the site of the tumour in purified or humanised form. The dose rate for the CD40 depending upon whether it is a monoclonal antibody or CD40L and may lie in the microgram to milligram range.

Pharmaceutically acceptable carriers (diluents, excipients, etc.) which are used with the CD40 binder can be selected from any of the known carriers for these types of drug and will depend, inter alai, upon the mode of administration. For example, for producing an injectable composition, the carrier may be selected from simple preparations such as phosphate-buffered saline containing human serum albumin to more complex solutions such as cell culture media.

The present invention will now be described in further detail in the following examples and with reference to the accompanying figures, in which:-

Fig 1 is a chart showing the effect of 48 hours treatment with anti-CD40 mAb G28.5 on the growth of ovarian carcinoma cell line A2780, the data representing the mean of triplicate determinations from three independent experiments and being presented as % decrease in survival relative to untreated controls,

Fig 2 is a chart showing that pre-incubation of 2780CP cell line with 150 U/ml IFN-γ for 24 hours induces a 26% decrease in cell growth in the presence of 2 μg/ml G28.5 mAb,

Fig 3 is a chart showing the effect of time and concentration of G28.5 mAb on the cell growth of an EJ bladder carcinoma cell line,

Fig 4 is a chart showing the effect of anti-CD40 mAb on the growth of Rat-1/CD40 transfectant (Cl.8),

Fig 5 is a chart showing the enhancement of cytotoxicity by the combination of G28.5 mAb and 5 μM *cis*-platin in the ovarian carcinoma cell line A2780, cytotoxicity being measured in a 48 hour MTT assay, the data representing the mean of triplicate determinations from three independent experiments and being presented as % decrease in survival relative to untreated controls,

Fig 6 is a chart showing the enhancement of *cis*-platin-induced cytotoxicity in an EJ bladder carcinoma cell line by incubation for 48 hours with 2 μg/ml G28.5 mAb,
Fig 7 is a chart showing the effect of the incubation of Rat-1/CD40 Cl.8 cells with 1μg/ml G28.5 mAb on various concentrations of *cis*-platin, the data representing the mean of triplicate determinations from three independent experiments,
Fig 8 is a chart showing the effect of 48 hours treatment with CD40L on the growth of the ovarian carcinoma cell line A2780, the data representing the mean of triplicate determinations from three independent experiments and being presented as % decrease in survival relative to untreated controls,
Fig 9 is a chart showing the effect of time and concentration of CD40L on the cell growth of an EJ bladder adenocarcinoma cell line,
Fig 10 is a chart showing the effect of CD40L on the growth of a Rat-1/CD40 transfectant (Cl.8), and
Fig 11 is a chart showing the enhancement of *cis*-platin-induced cytotoxicity in the EJ bladder carcinoma cell line by incubation for 48 hours with 100 U/ml CD40L.

Example 1

Cell culture

A human ovarian carcinoma cell line A2780 and its *cis*-platin-resistant derivative 2780CP (see Masuda et al., Cancer Res., **50**, 1863-1866, 1990), an EJ bladder carcinoma cell line (Accession No.85061108, European Collection of Animal Cell Cultures, Porton Down, UK) and Rat-1 fibroblasts (Lania et al., Virology, **101**, 217-232, 1980) were continuously maintained in RPMI 1640 medium (GIBCOBRL, Life technologies Ltd, Paisley, Scotland) supplemented with 10% FCS (foetal

calf serum - ICN Flow, Thane, Oxfordshire, England), 2mM glutamine, and the antibiotics, penicillin (1000 U/ml) and streptomycin (1mg/ml) (Sigma Co. Ltd, Poole, Dorset, England) at 37°C in a 5% CO_2 atmosphere.

Rat-1 fibroblasts expressing CD40 were generated by electroporation of the CD40 expression vector (following the procedure described by Stamenkovic et al., EMBO J., 8, 1403-1410, 1989) with a suitable drug resistant plasmid, pUC LTR neo, at a ratio of 10:1. Stable transfectants of Rat 1 cells expressing CD40 were isolated after prolonged exposure to Geneticin G418 antibiotic at which time individual colonies of drug-resistant cells were harvested and separately cultured as individual colonies. Samples of such transfectants have been deposited under the provisions of the Budapest Treaty with European Collection of Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, Wiltshire SP4 0JG, Great Britain, on 10 November 1995 under the Accession No. 951110100.

Expression of the CD40 in the above cell lines was confirmed with FACS analysis using the anti-CD40 antibody, G28.5 mAb. Briefly, cells were harvested after trypsinisation, washed in phosphate-buffered saline (PBS) and resuspended in 0.5 μg/ml G28.5 mAb. After 1 hour incubation at 4°C, the cells were washed in PBS and resuspended in 1:50 dilution of FITC-conjugated goat anti-mouse IgG (Sigma Chemical Co. Ltd, Poole, Dorset, England). After a further 30 minute incubation at 4°C, the cells were washed and finally resuspended in 1% paraformaldehyde prior to standard FACS analysis.

Treatment of cells and Assay for Cell Growth

Inhibition of cell proliferation was evaluated using the colorimetric assay of Mossman (see Mossman, J. Immunol. Meths., **65**, 55-63, 1983). In brief, cells were plated on a 96 well plate overnight to enter normal cell cycle. Various concentrations of G28.5 mAb were then added and their effect on cell growth was evaluated after an appropriate period of time (48, 72 or 96 hrs) by the addition of 20 μl of 5 mg/ml MTT (3-(4,4-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) obtained from Sigma, in PBS. The cells were further incubated for 5 hrs at 37°C, formazan crystals thus formed were dissolved in DMSO and the optical density (O.D.) was recorded at 550nm on a Becton Dickinson Multiscan. In a further series of experiments, cells were treated for 2 hrs with various *cis*-platin concentrations with or without G28.5 mAb. The cells were then washed twice in PBS and 200μl of fresh medium containing G28.5 mAb. After 48 hours, cell growth was assessed using the MTT assay as described above.

Effect of anti-CD40 mAb on carcinoma cell growth

The effect of the anti-CD40 mAb G28.5 on the cell growth of the A2780, 2780CP and EJ carcinoma cell lines as well as a CD40 Rat-1 transfectant (Rat-1/CD40 Cl.8) was examined. G28.5 mAb at the optimum concentration of 10 μg/ml induced a 29% decrease in survival of the ovarian A2780 cell line relative to control after 48 hrs treatment (Fig.1).

When the *cis*-platin-resistant 2780CP cell line was treated under similar conditions with G28.5 mAb, no effect was observed. This was probably due to the low level of CD40 expression in this cell line. However, treatment of the 2780CP cell line with 150 U/ml of interferon-γ(IFNγ) for 24 hrs and subsequent incubation with 10 μg/ml G28.5, induced an approximately 22% decrease in survival consistent with the induction of

CD40 by IFNγ (Fig. 2). Similar effects of CD40 activation were observed with the EJ bladder carcinoma cell line (Fig. 3). The effect of G28.5 mAb on the EJ cell line is both time- and concentration-dependent with maximum effect at 96 hrs where treatment with 2 μg/ml G28.5 induced a 27% decrease in survival (Fig. 3).

The specificity of the CD40 effect was investigated using Rat-1 fibroblasts expressing a transfected CD40. As demonstrated in Fig. 4, stimulation of CD40 by the G28.4 mAb inhibited cell growth. In the transfectant studied (clone 8), incubation for 48 hrs with 2 μg/ml G28.5 mAb induce a 22% decrease in cell growth (Fig. 4).

Enhancement of cis-platin-induced cell death by CD40 activation
Treatment of the above cell lines with G28.5 mAb enhanced the cytotoxic effect of anti-cancer drugs such as *cis*-platin. In the A2780 cell line, exposure to 10 μg/ml G28.5 mAb increased the cell killing effect of 5 μM *cis*-platin from 46% to 61% (Fig. 5), which represents a statistically significant effect. Similar results were obtained with the EJ line treated with 5 μM *cis*-platin in the presence of 2 μg/ml G28.5 mAb (Fig. 6).

Treatment of the Rat-1/CD40 Cl.8 cell line with 1 μg/ml G28.5 mAb also enhanced the cytotoxic effect of *cis*-platin by shifting the IC50 value from 6 μM (+/- 0.9) to 4.2 μM (+/- 0.3), representing a 1.6-1.8 fold increase in drug sensitivity (Fig 7). No effect was found in parental untransfected Rat-1 cells).

Example 2

Cell culture

The cell culture techniques described hereinabove in Example 1 were utilised.

Treatment of cells and Assay for Cell Growth

Inhibition of cell proliferation was evaluated using the colorimetric assay of Mossman (Mossman, J. Immunol. Meths. 65, 55-63, 1983). In brief, cells were plated on a 96 well plate overnight to enter normal cell cycle. Various concentrations of CD40L (an engineered soluble form of a CD40 binding ligand (Immunex Research and Development Corporation, 51 University Street, Seattle, Washington, USA) were then added and their effect on cell growth was evaluated after the appropriate period of time (48, 72 or 96 hrs) by the addition of 20 μl of 5 mg/ml MTT (3-(4,4-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) obtained from Sigma, in PBS. The cells were further incubated for 5 hrs at 37°C, formazan crystals thus formed were dissolved in DMSO and the optical density (O.D.) was recorded at 550nm on a Becton Dickinson Multiscan. In another series of experiments, cells were treated for 2 hrs with various *cis*-platin concentrations with or without CD40L. The cells were then washed twice in PBS and 200μl of fresh medium containing CD40L. After 48 hours, cell growth was assessed using the MTT assay as described above.

Effect of CD40L on carcinoma cell growth

The effect of the CD40L on the cell growth of the A2780, 2780CP and EJ carcinoma cell lines as well as a CD40 Rat-1 transfectant (Rat-1/CD40 Cl.8) was examined. 100 U/ml of CD40L induced a 36% decrease in survival of the ovarian A2780 cell line relative to control after 48 hrs treatment (Fig. 8). The effect of CD40L on the EJ cell line is both time and concentration-dependent with maximum effect at 96 hrs where

treatment with 100 U/ml CD40L induced a 43% decrease in survival (Fig. 9).

The specificity of the CD40 effect was investigated using Rat-1 fibroblasts expressing a transfected CD40. As demonstrated in Fig. 10, stimulation of CD40 by CD40L inhibited cell growth. In the transfectant studied (clone 8), incubation for 48 hrs with 100 U/ml CD40L induced a 28% decrease (Figure. 10).

Enhancement of cis-platin-induced cell death by CD40 activation
Treatment of the EJ carcinoma cell line with 7.5 μM *cis*-platin in the presence of 100 U/ml of CD40L showed enhancement of cell death (Figure 11).

CLAIMS

1. The use of a CD40 receptor binder for the manufacture of a medicament for the prevention of epithelial cell proliferation or for the treatment of carcinomas in epithelial cell systems.

2. The use of a CD40 receptor binder in the manufacture of a medicament for enhancing the susceptibility of neoplastic epithelial cells to anti-neoplastic drug-induced apoptosis.

3. The use of a CD40 receptor binder in combination with a CD40 inducer for the prevention of epithelial cell proliferation or for the treatment of carcinomas in epithelial cell systems.

4. The use of a CD40 receptor binder in combination with an anti-neoplastic drug for the treatment of carcinomas in epithelial cell systems.

5. The use as claimed in any preceding claim, wherein the CD40 receptor binder is selected from ligand mimetic monoclonal antibodies and naturally occuring or engineered forms of CD40 ligands.

6. The use as claimed in any one of claims 1 to 4, wherein the CD40 receptor binder is selected from G28.5 and CD40L.

7. A method of preventing epithelial cell proliferation, comprising administering a CD40 receptor binder to a human or animal patient.

8. A method of treating an epithelial cell carcinoma in a human or animal patient, comprising administering a CD40 receptor binder to the patient.

9. A method as claimed in claim 7 or 8, wherein administration of the CD40 receptor binder is effected in conjunction with administration of at least one anti-neoplastic agent and/or a CD40 inducer.

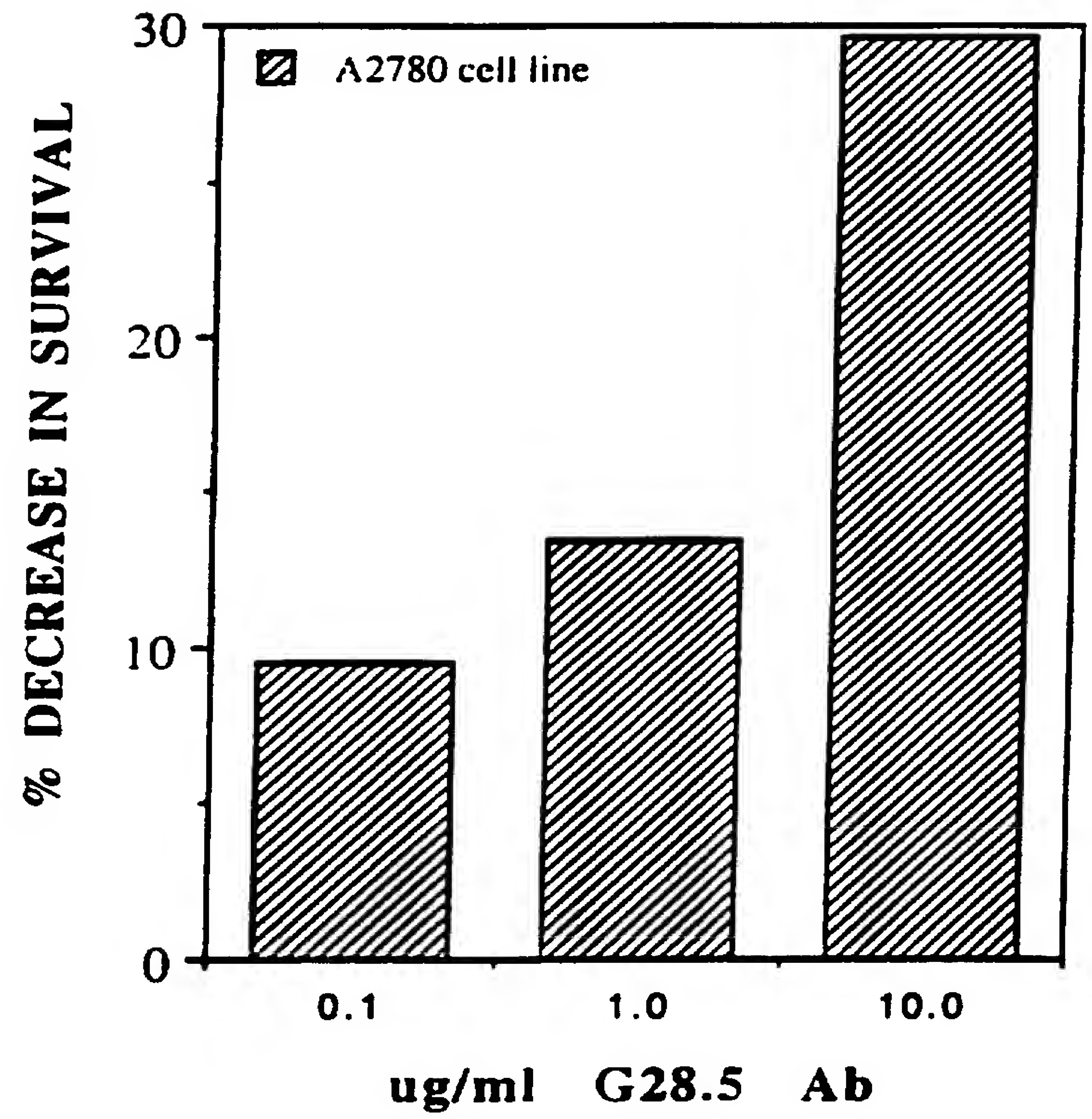
30
20
10
0
A2780 cell line
% DECREASE IN SURVIVAL
0.1
1.0
10.0
ug/ml G28.5 Ab

FIG 1

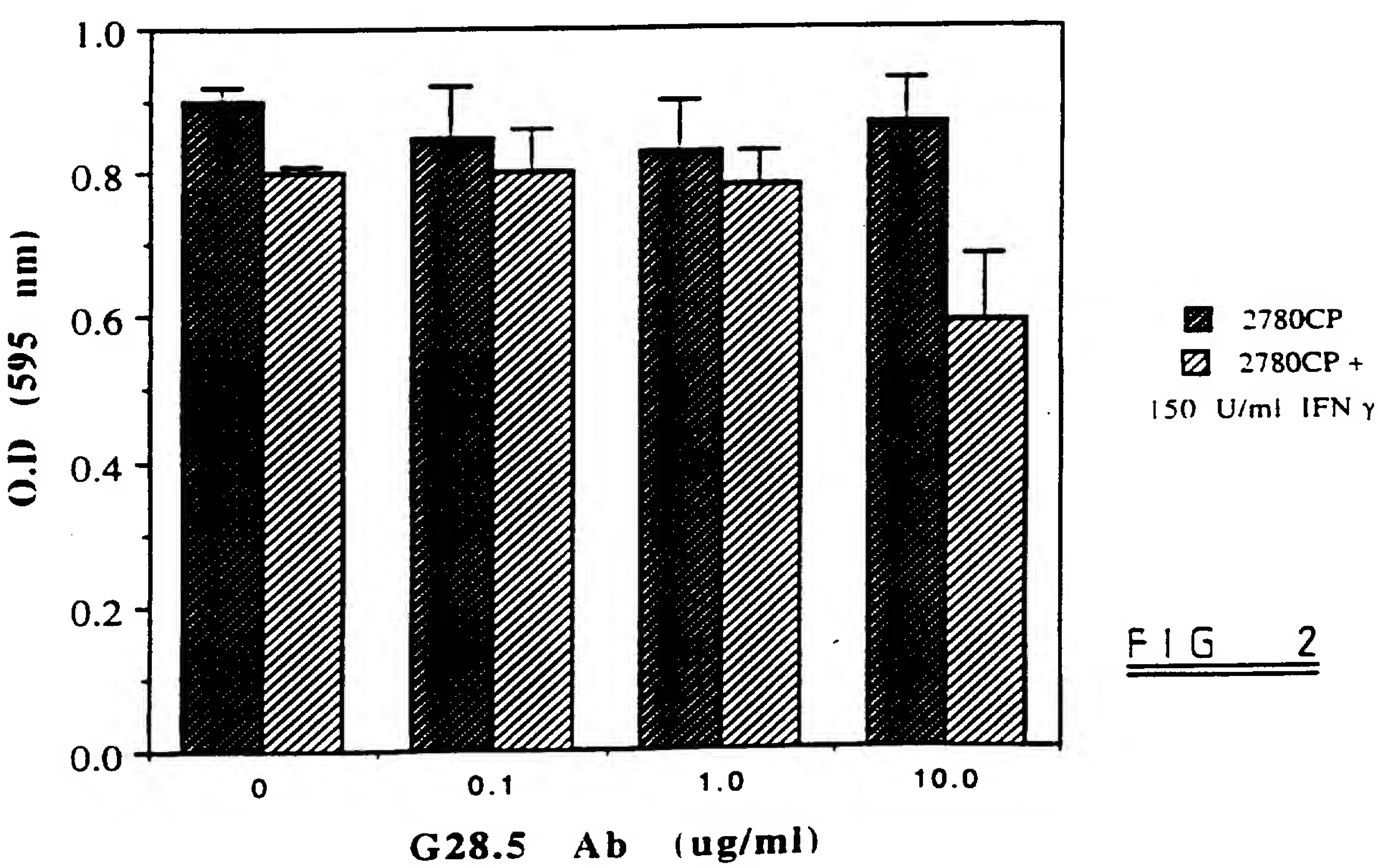
1.0
0.8
0.6
0.4
0.2
0.0
O.D (595 nm)
0
0.1
1.0
10.0
G28.5 Ab (ug/ml)
2780CP
2780CP +
150 U/ml IFN γ

FIG 2

EJ bladder carcinoma cell line

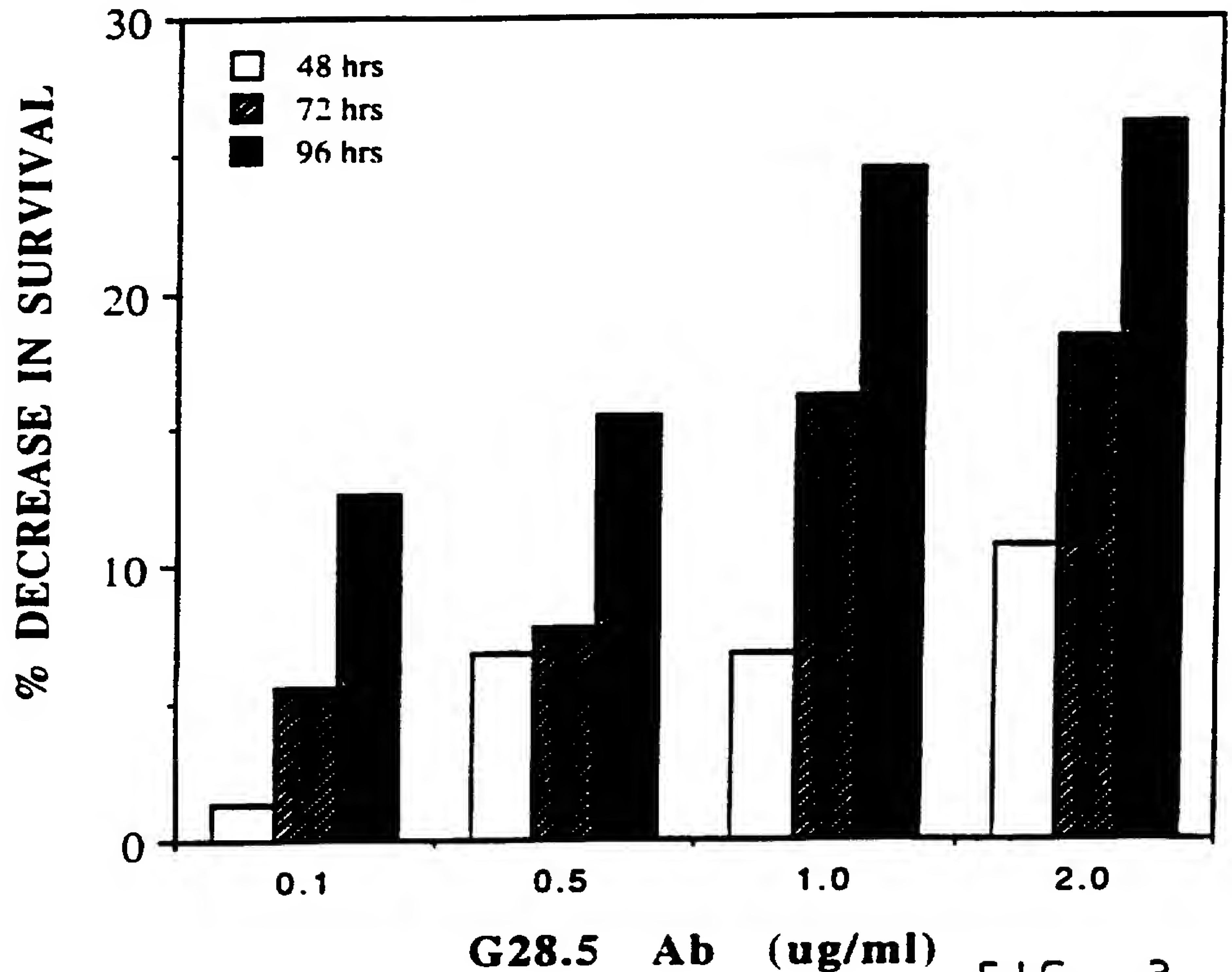

FIG 3

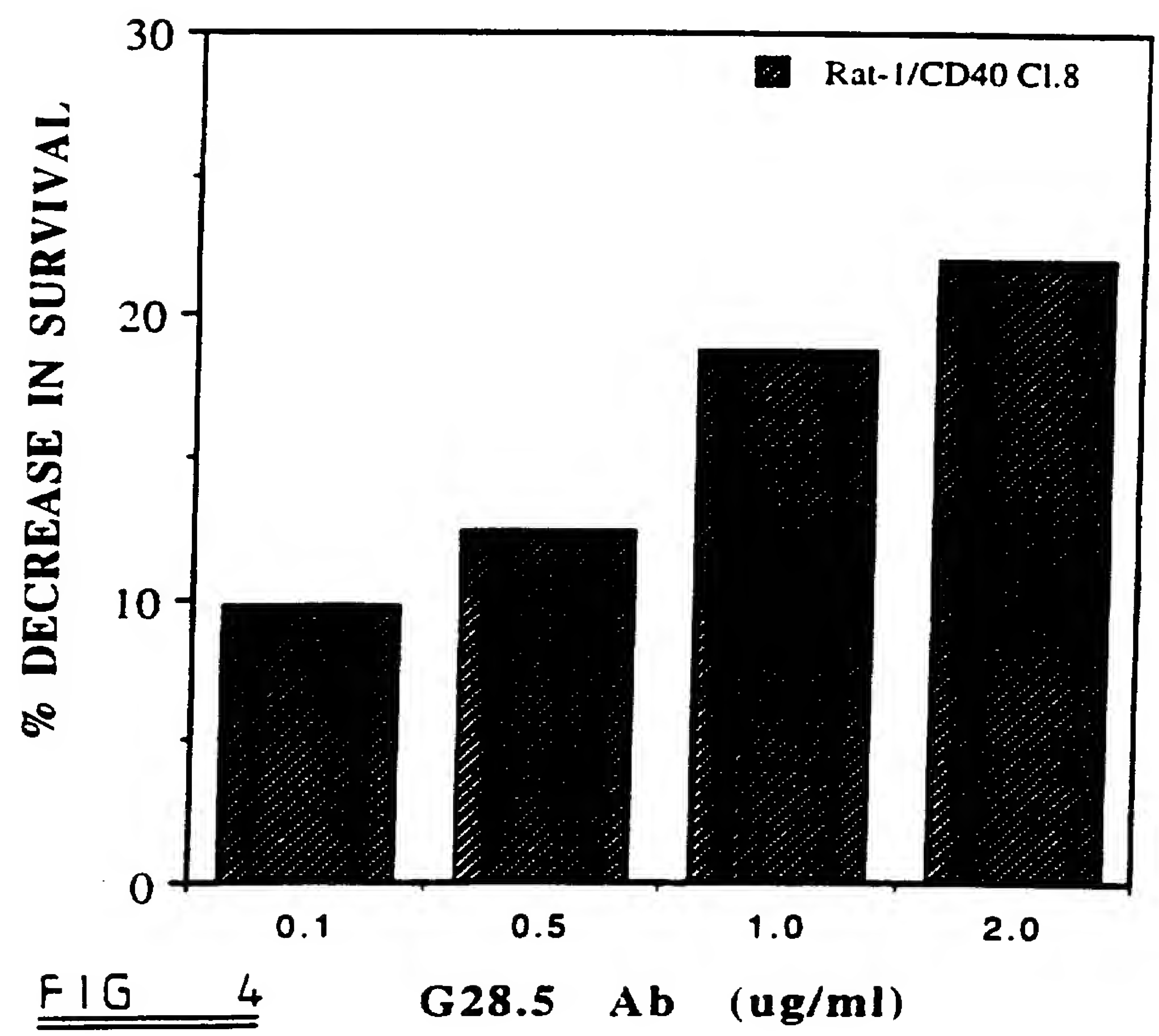

FIG 4

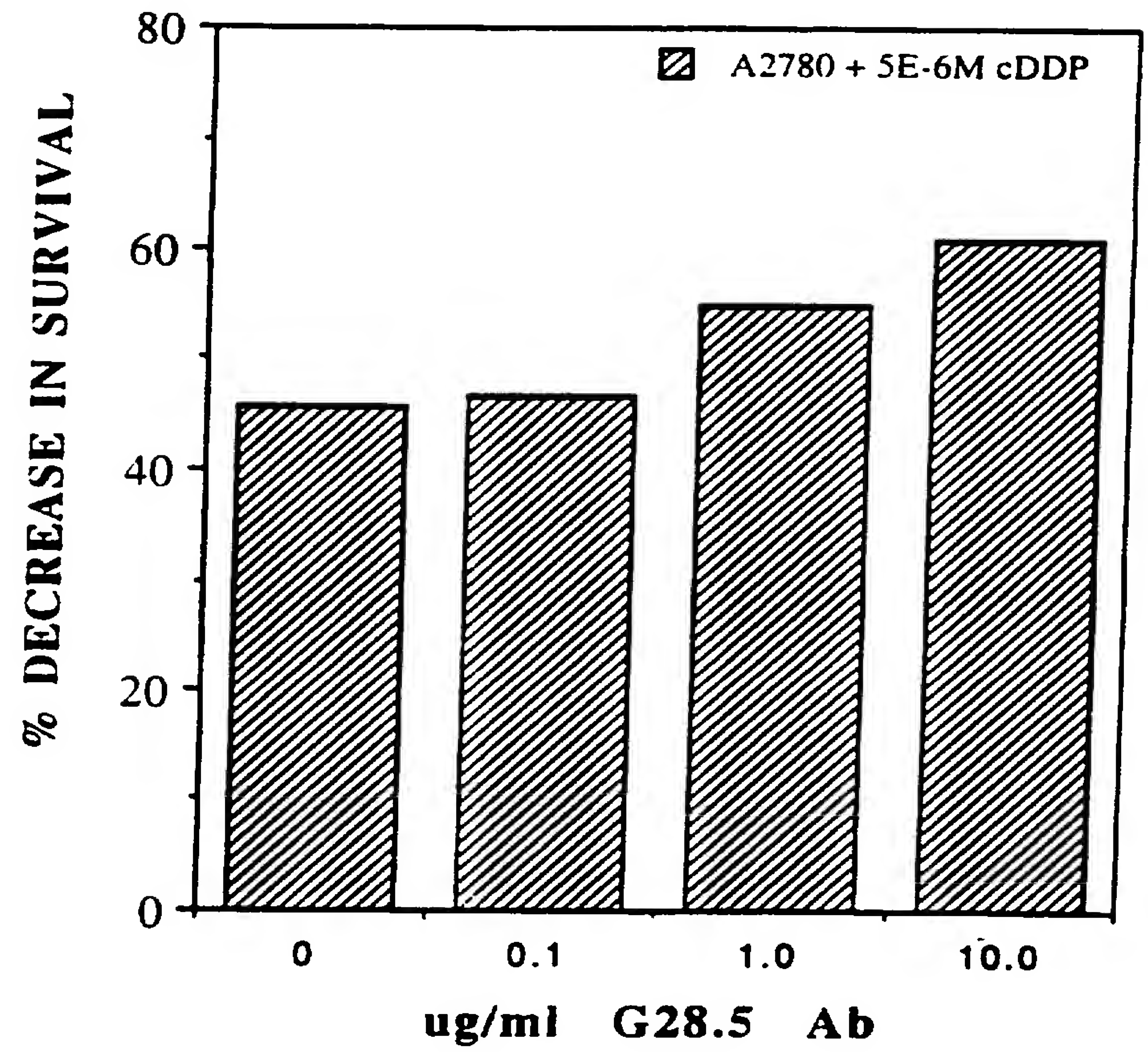
A2780 + 5E-6M cDDP
% DECREASE IN SURVIVAL
80
60
40
20
0
0
0.1
1.0
10.0
ug/ml G28.5 Ab

FIG 5

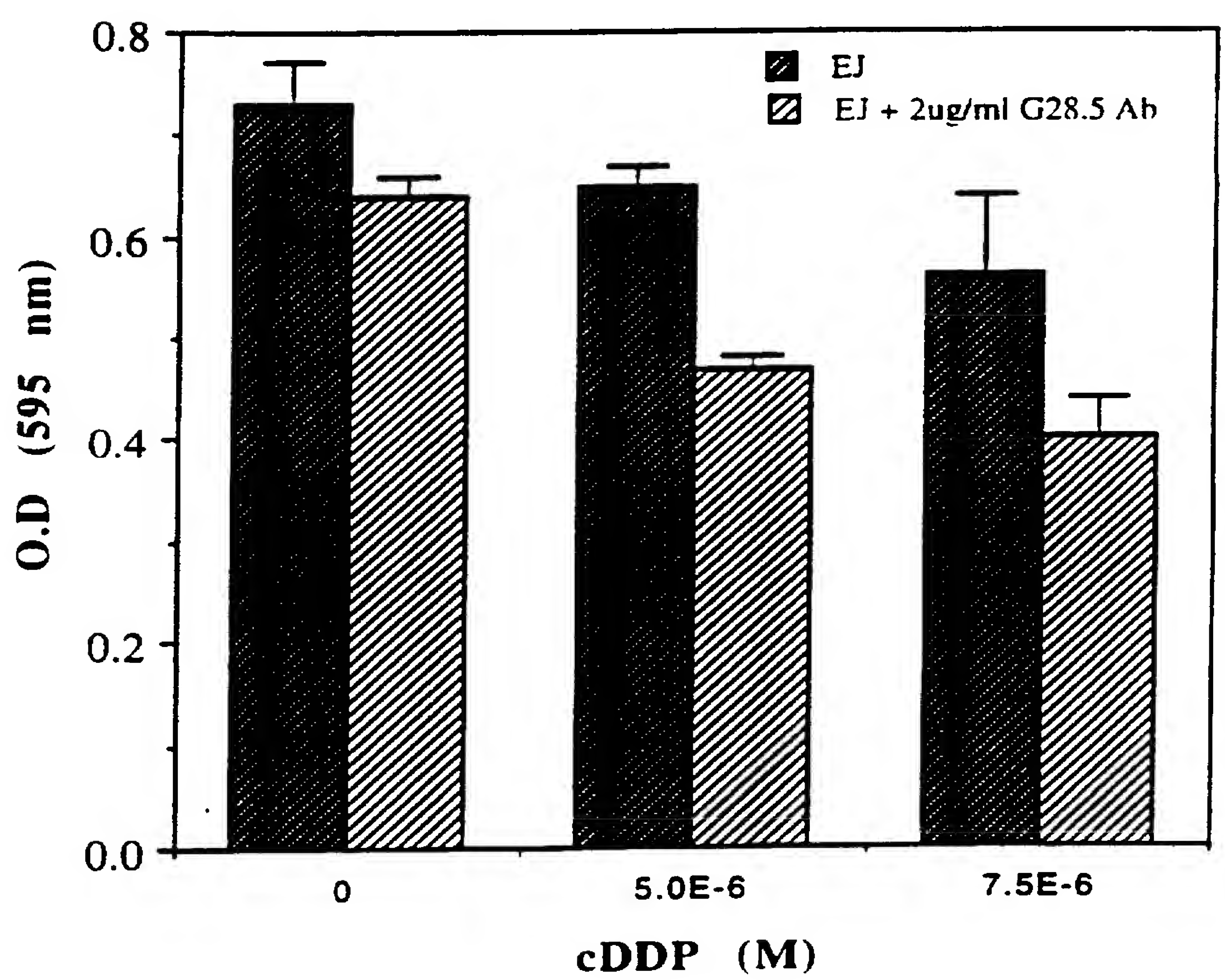
EJ
EJ + 2ug/ml G28.5 Ab
O.D (595 nm)
0.8
0.6
0.4
0.2
0.0
0
5.0E-6
7.5E-6
cDDP (M)

FIG 6

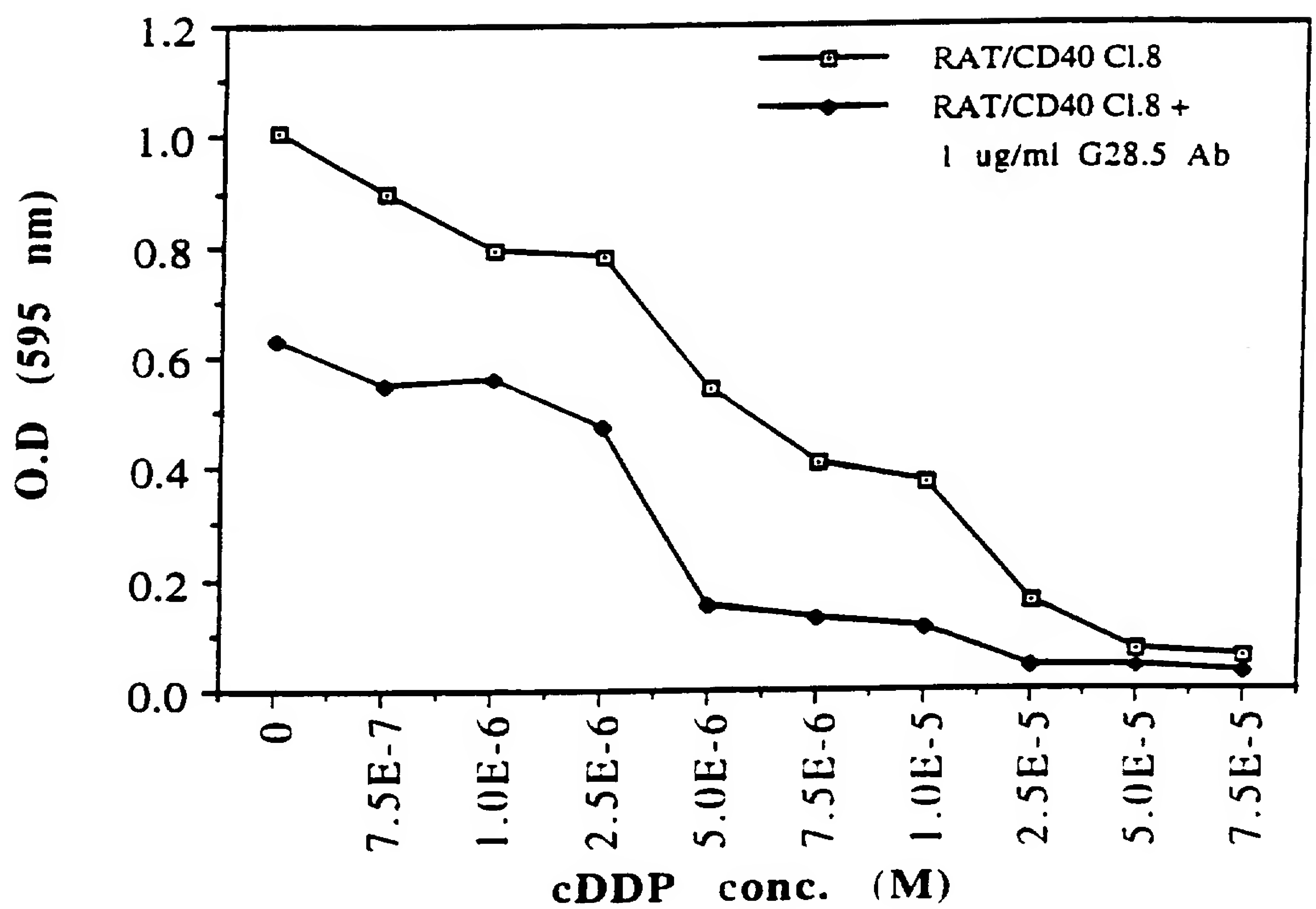
1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D (595 nm)
RAT/CD40 Cl.8
RAT/CD40 Cl.8 +
1 ug/ml G28.5 Ab
0
7.5E-7
1.0E-6
2.5E-6
5.0E-6
7.5E-6
1.0E-5
2.5E-5
5.0E-5
7.5E-5
cDDP conc. (M)

FIG 7

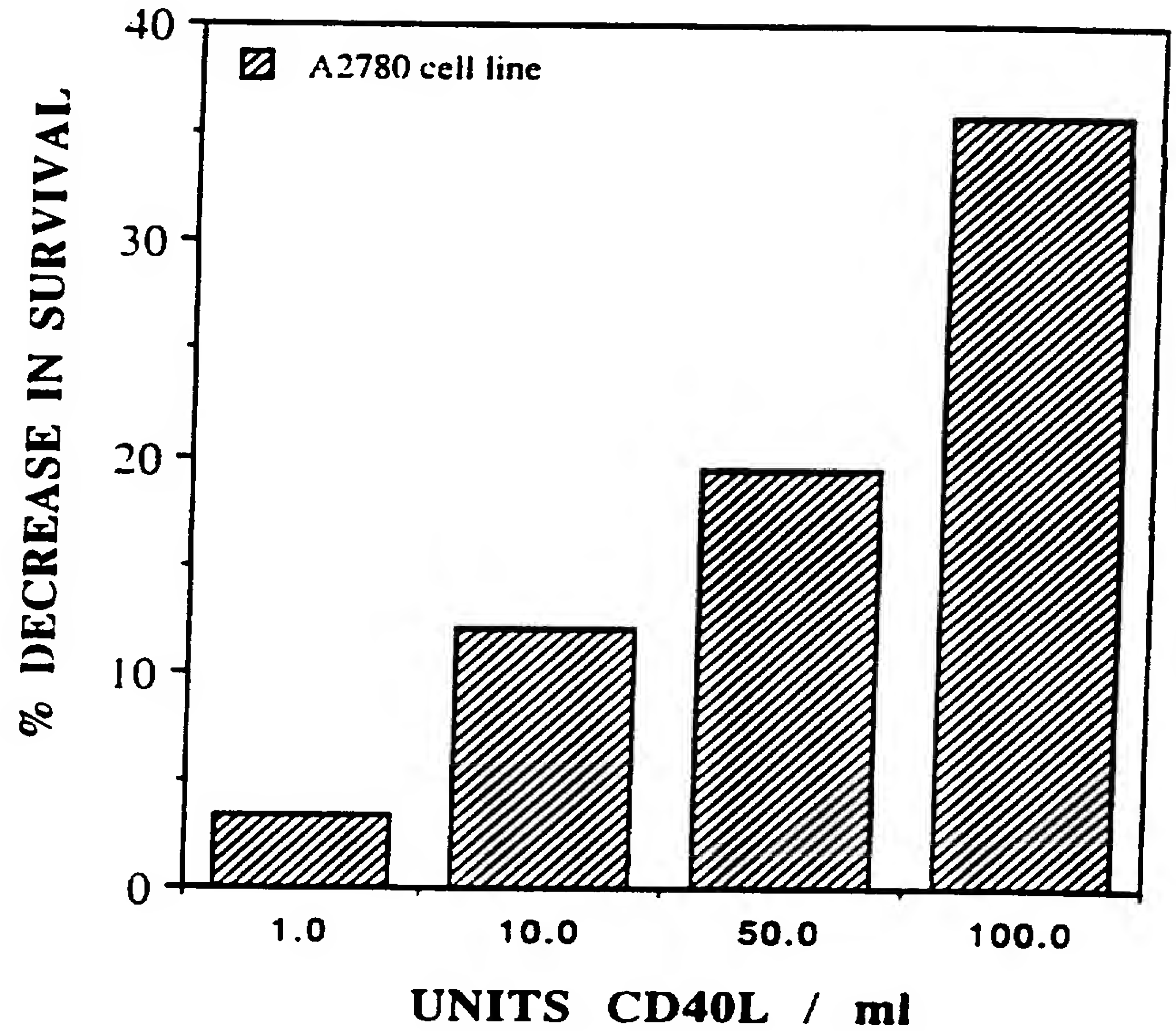
40
30
20
10
0
% DECREASE IN SURVIVAL
A2780 cell line
1.0
10.0
50.0
100.0
UNITS CD40L / ml

FIG 8

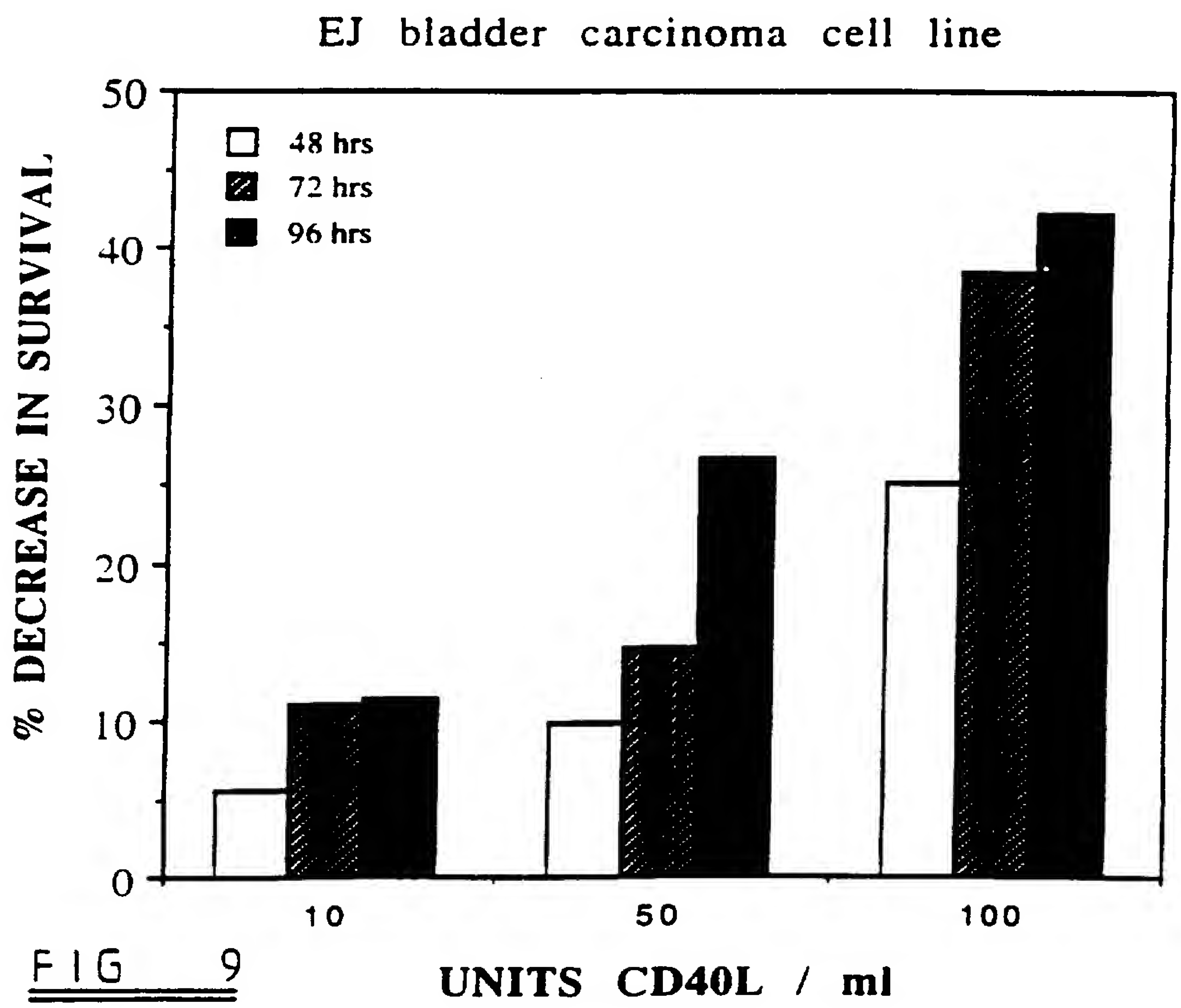
EJ bladder carcinoma cell line
50
40
30
20
10
0
% DECREASE IN SURVIVAL
48 hrs
72 hrs
96 hrs
10
50
100
UNITS CD40L / ml

FIG 9

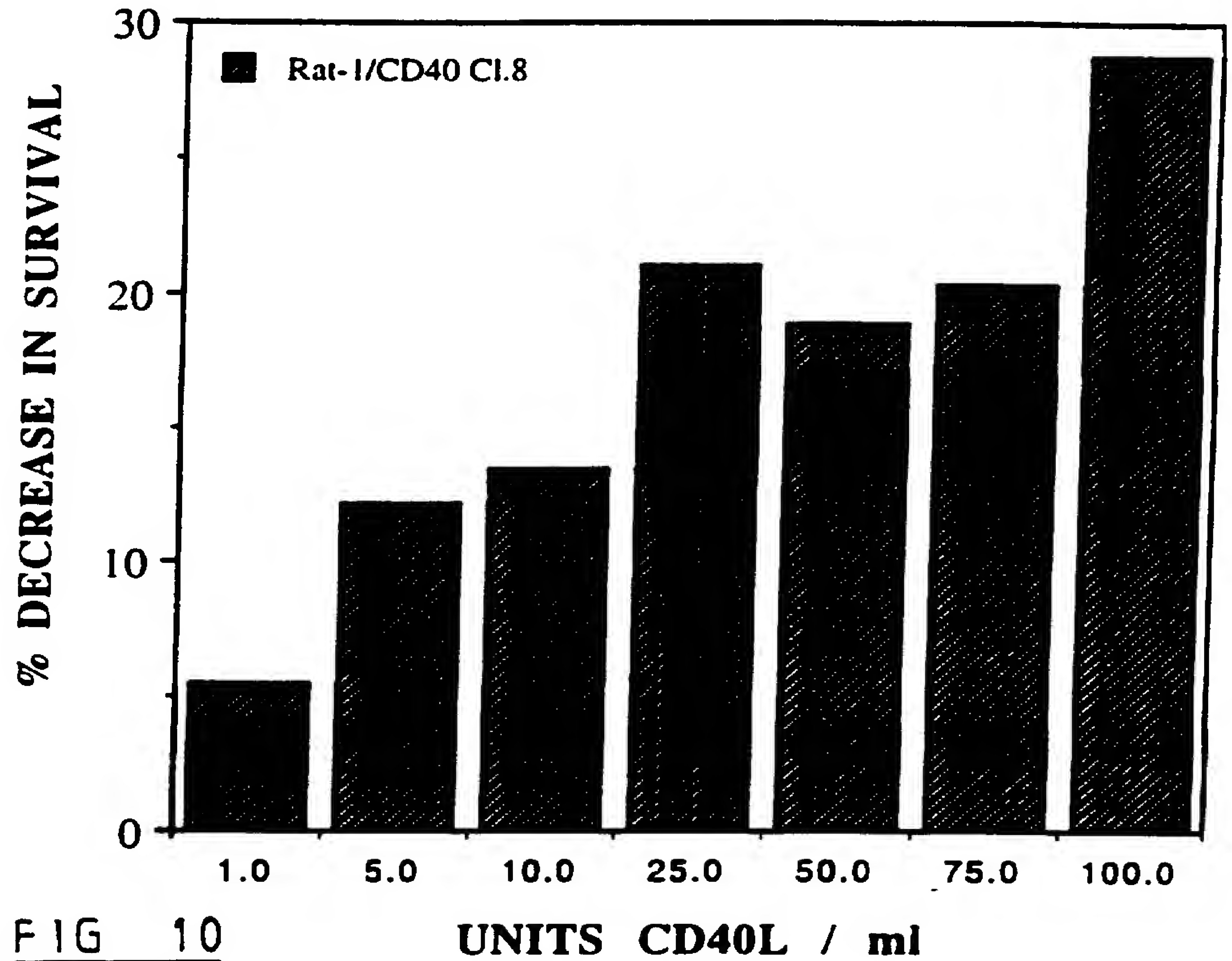
30
20
10
0
% DECREASE IN SURVIVAL
Rat-1/CD40 Cl.8
1.0
5.0
10.0
25.0
50.0
75.0
100.0
UNITS CD40L / ml

FIG 10

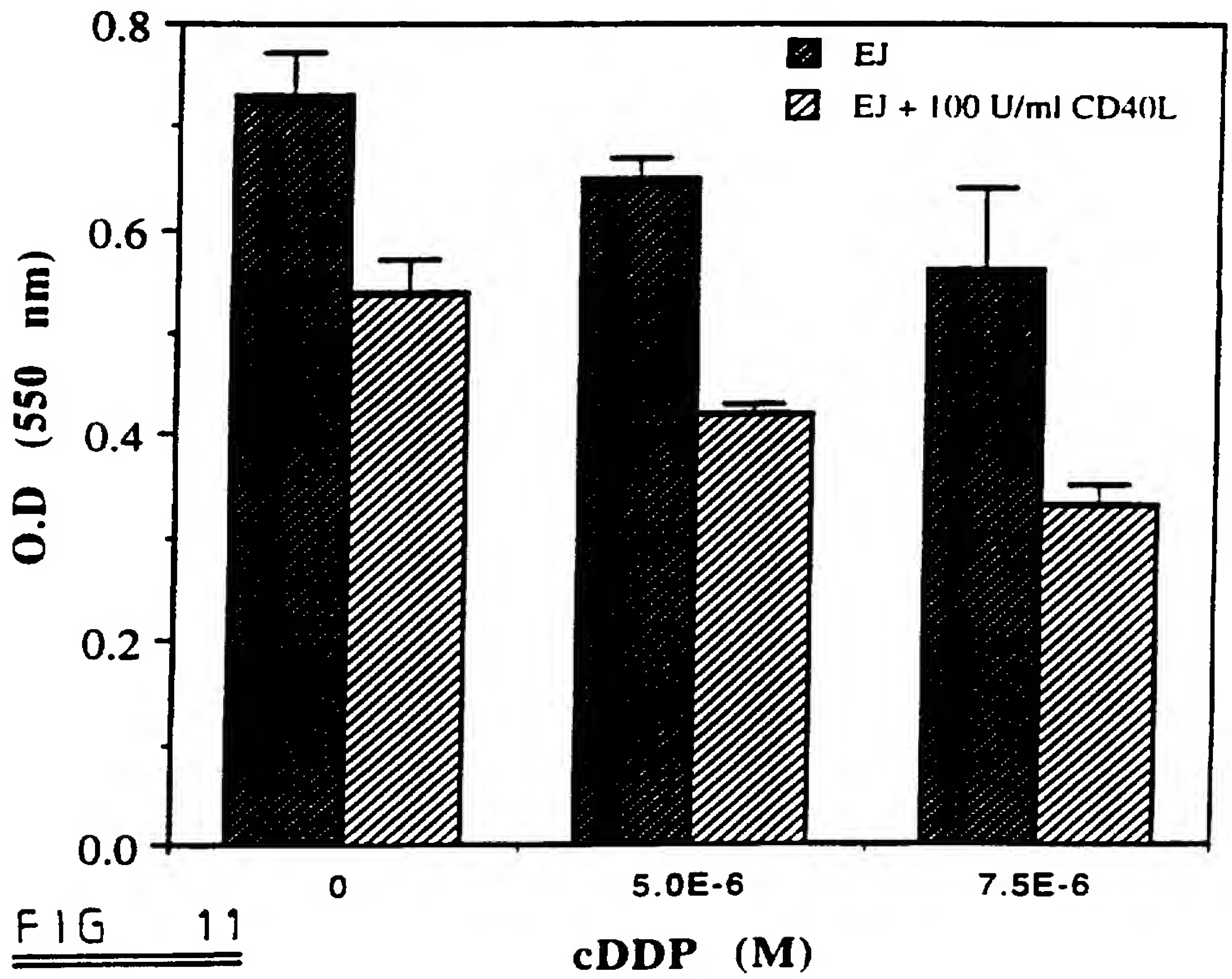
0.8
0.6
0.4
0.2
0.0
O.D (550 nm)
EJ
EJ + 100 U/ml CD40L
0
5.0E-6
7.5E-6
cDDP (M)

FIG 11

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO
CRC Institute for Cancer Studies
University of Birmingham
Medical School
Birmingham, B15 2TH

NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: CRC Institute for Cancer Studies Address: University of Birmingham Medical School Birmingham B15 2TH	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: 951110100 Date of the deposit or of the transfer:[1] 10th November 1995

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 10/11/95 [2]. On that date, the said microorganism was

[X] [3] viable

[ ] [3] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

2 5 JAN 1996

INTERNATIONAL FORM

TO
CRC Institute for Cancer Studies
University of Birmingham
Medical School
Birmingham, B15 2TH

NAME AND ADDRESS OF DEPOSITOR

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Rat - 1/CD40 Cl.8	951110100

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[X] a scientific description

[ ] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 10/11/95 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Dr A Doyle

Address: ECACC, CAMR, Porton Down Salisbury, Wilts, SP4 OJG

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 15.1.96

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Dr A Doyle Address: ECACC, CAMR, Porton Down Salisbury, Wilts, SP4 OJG	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 10.1.96

[4] Fill in if the information has been requested and if the results of the test were negative.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/GB 95/02807

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 A61K39/395 A61K38/18 C07K14/475

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	BLOOD, vol. 86 (10 SUPPL. 1), 1995 page 318A, Document 1261 M. A. DEGLI-ESPOSTI ET AL. 'CD40 ligand, a potent growth factor, causes apoptosis of bladder carcinoma cell lines.' see the whole document ---	1-9
Y	EP,A,0 585 943 (SQUIBB BRISTOL MYERS CO) 9 March 1994 see page 2, line 57 - page 3, line 13; claim 32 --- -/--	1-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 1996	16.04.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Halle, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	IMMUNOLOGY, vol. 79, no. 3, 1993 pages 445-451, L. FLORES-ROMO ET AL. 'Anti-CD40 antibody stimulates the VLA-4-dependent adhesion of normal and LFA-1-deficient B cells to endothelium' see page 447, left column, line 10 - right column, line 7	1-9
A	NATURE, vol. 357, no. 6373, 1992 pages 80-82, RICHARD J. ARMITAGE ET AL. 'Molecular and biological characterization of a murine ligand for CD40' see the whole document	
A	INT. J. CANCER, vol. 43, 1989 pages 786-794, L. S. YOUNG ET AL. 'Identification of a human epithelial cell surface protein sharing an epitope with the C3d/Epstein-Barr virus receptor molecule of B lymphocytes' cited in the application see page 791, left column, paragraph 2	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 95/02807

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0585943	09-03-94	AU-B- 4612093	10-03-94
		CA-A- 2105552	05-03-94
		FI-A- 933862	05-03-94
		HU-A- 69977	28-09-95
		JP-A- 6315383	15-11-94
		NO-A- 933126	07-03-94
		NZ-A- 248569	26-10-95
		ZA-A- 9306491	25-03-94